Potential intercellular futile cycling of carbohydrates in diabetes

Nancy W. KLECKNER, Zenro KIZAKI and Ronald G. THURMAN*
Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, U.S.A.

Diabetes was induced by treating rats with alloxan, and was confirmed by blood glucose values greater than 250 mg/dl. In perfused livers from both normal and diabetic rats, basal rates of O_2 uptake were similar (120–130 μmol/h per g). In livers from diabetic rats, basal rates of glucose output of 60 μmol/h per g declined to around 20 μmol/h per g during 1 h of perfusion. Basal glucose production was abolished by pretreatment with an inhibitor of glycogen synthesis, galactosamine (1.5 g/kg), injected 3 h before perfusion. The subsequent infusion of lactate (2 mM) increased O_2 uptake and glucose production about 40–50 μmol/h per g in both groups; however, the average maximal increase in glucose output was nearly twice as high in livers from normal (33 μmol/h per g) as from diabetic (18 μmol/h per g) rats. Rates of lactate uptake were also about 50% lower in livers from diabetic than from normal rats, yet rates of ketone-body formation were similar. Miniature O_2 electrodes placed on periportal and pericentral regions of the liver lobule were employed to measure local rates of O_2 uptake before, during and after infusion of lactate by stopping the flow of perfusate through the liver and measuring the decrease in local [O_2]. Local rates of glucose production were calculated from the extra O_2 consumed and the known stoichiometry between O_2 uptake and glucose production from lactate. In livers from normal rats, glucose was synthesized predominantly in periportal regions of the liver lobule; however, glucose was produced exclusively in periportal regions in livers from diabetic rats. In pericentral regions, O_2 uptake increased slightly in livers from normal rats, but declined significantly by 10 μmol/h per g in livers from diabetic rats. These data are consistent with the hypothesis that gluconeogenesis from lactate occurs exclusively in periportal regions of the liver lobule in livers from diabetic rats. A portion of this glucose is metabolized back to lactate in pericentral areas, leading to increased rates of glycolytic ATP production, thereby decreasing the demands for O_2. This production of glucose from lactate in periportal regions, followed by conversion of glucose back into lactate in pericentral areas, raises the possibility of intercellular futile cycling, stimulated by diabetes.

INTRODUCTION

Data have accumulated over the past decade demonstrating that cells in different regions of the hepatic lobule do not contain similar amounts and activities of many key enzymes (Rapaport, 1976; Jungermann & Katz, 1982). Using micro-dissection techniques, Guder and co-workers (Guder & Schmidt, 1976) demonstrated quantitative differences in many enzyme activities in tissues from periportal and pericentral zones of the liver lobule. Major enzymes involved in gluconeogenesis are localized preferentially in periportal regions of the lobule (Babcock & Candell, 1974; Sasse *et al.*, 1975), whereas many glycolytic enzymes predominate in pericentral areas (Sasse *et al.*, 1975; Hildebrand, 1980). On the basis of these differences, Jungermann and his colleagues suggested that gluconeogenesis is localized in periportal regions and that glycolysis predominates in pericentral areas of the liver lobule (Jungermann *et al.*, 1977; Jungermann & Katz, 1982). In spite of this information, flux of carbohydrate in the gluconeogenic pathway in intact cells located in periportal and pericentral regions cannot be deduced solely from differences in maximal activity of a few enzymes. Substrate concentrations and rates of cofactor supply, as well as local concentrations of intracellular modulators, could also be important. Therefore methods were developed to measure glucose synthesis in specific regions of the liver lobule experimentally (Matsumura *et al.*, 1984; Thurman & Kauffman, 1985; Lemasters *et al.*, 1986). To determine metabolic flux rates in different regions of the liver lobule, miniature O_2 electrodes have been used to measure changes in rates of O_2 uptake in periportal and pericentral regions when substrate (e.g. lactate) was infused into livers from starved rats, perfused in the anterograde direction (Matsumura & Thurman, 1983, 1984; Matsumura *et al.*, 1984). Glucose production was calculated from the extra O_2 uptake measured with miniature O_2 electrodes and the known relationships between O_2 uptake, ATP synthesis and the energy demands for gluconeogenesis. Such studies demonstrated that gluconeogenesis predominates in periportal regions of the liver lobule. When livers were perfused in the retrograde direction, however, maximal gluconeogenic activities were switched rapidly to cells in the opposite region of the lobule (Matsumura & Thurman, 1984; Matsumura *et al.*, 1984). Thus factors other than maximal enzyme activity appear to be important factors in the short-term regulation of hepatic gluconeogenesis.

Hormones also have differential effects on cells in different zones of the hepatic lobule. Adrenaline stimulated gluconeogenesis about 3-fold more in pericentral than in periportal regions of the liver lobule in perfusions in the anterograde direction (Matsumura *et al.*, 1984). In contrast, glucagon stimulated glucose synthesis exclusively in periportal regions in perfusions in

* To whom reprint requests should be addressed.

Table 1. Estimation of fluid volume of perfused livers from normal and diabetic rats

Values are means ± S.D. for the numbers of rats shown in parentheses, treated as described in the legend to Fig. 2. Livers from rats that were treated with vehicle or were made diabetic were perfused with Krebs–Henseleit bicarbonate buffer. The flow was stopped, followed by the ligation of portal vein. The liver was then detached and weighed (weight of liver with fluid). The ligatures on the portal vein were removed and the liver was blotted dry and reweighed (wet weight). The dry weight of the liver was obtained after 48 h of drying (70 °C). The fluid content (intra- and extra-cellular) was calculated by the formula (wt. of liver with fluid − liver dry wt.)/liver wet wt.

Treatment	Rat wt. (g)	Liver wt. (g)				Fluid–dry
		With fluid	Wet	Dry	Wet/dry	wet
None (5)	181 ± 12	9.1 ± 0.8	8.1 ± 0.8	2.1 ± 0.2	3.8 ± 0.1	0.87 ± 0.03
Diabetes (6)	148 ± 14	8.8 ± 1.2	7.8 ± 1.1	2.0 ± 0.3	3.9 ± 0.1	0.87 ± 0.02

the anterograde direction (Kinugasa & Thurman, 1986). Interestingly, the lobular site of action of both hormones was switched rapidly to cells in the opposite end of the lobule when the direction of perfusion was reversed (Matsumura *et al.*, 1984; Kinugasa & Thurman, 1986).

Diabetes is characterized by a high glucagon/insulin ratio and high glucose concentrations in blood. Glucose production from lactate and other gluconeogenic precursors has been reported to be higher in diabetes in isolated hepatocytes (Wagle & Ingebretson, 1975), and activities of some gluconeogenic enzymes are higher in livers from diabetic rats (Wagle, 1964; Prinz & Seubert, 1964; Lardy *et al.*, 1965). These previous studies, however, did not provide direct information on the lobular distribution of gluconeogenesis in diabetes. The purpose of the present study was therefore to determine the effect of experimental diabetes on sublobular compartmentation of gluconeogenesis in the isolated perfused rat liver. Local rates of oxygen uptake were measured before, during and after lactate infusion by using a miniature O_2 electrode and the stopped-flow O_2-uptake technique (Matsumura & Thurman, 1983). The data indicate that gluconeogenesis from lactate occurs exclusively in periportal regions of the liver lobule in livers from diabetic rats. Moreover, the data are consistent with the hypothesis that newly synthesized glucose was reconverted into lactate in pericentral regions only in livers from diabetic rats. Preliminary accounts of this work have appeared elsewhere (Kleckner *et al.*, 1986).

MATERIALS AND METHODS

Animals and liver perfusion

Female Sprague–Dawley rats were maintained on lab chow *ad libitum*. They received sodium phenobarbital in drinking water (1 mg/ml) for 1–4 weeks before perfusion experiments, to enhance pigmentation differences between periportal and pericentral regions of the liver lobule (Ji *et al.*, 1980; Lemasters *et al.*, 1986).

Diabetes was produced by intravenous injection of alloxan monohydrate (120 mg/kg) in saline after starvation for 24 h. Blood samples were taken 48 h after injection and were analysed for glucose by standard enzymic procedures (Bergmeyer, 1974). Rats with blood glucose concentrations greater than 250 mg/dl were considered diabetic. At 2 days after injection of alloxan or vehicle, normal and diabetic rats were starved for 24 h before liver perfusion. All rats used in stopped-flow O_2-uptake experiments were injected intraperitoneally with galactosamine (1.5 g/kg) 3 h before perfusion to inhibit glycogen synthesis (Decker & Keppler, 1972). All livers from rats treated in this manner took up O_2 at similar rates to controls, and did not release lactate dehydrogenase or take up Trypan Blue, indicating that galactosamine treatment was not hepatotoxic under these conditions.

Livers were perfused with Krebs–Henseleit (1932) bicarbonate buffer (pH 7.4, 37 °C) saturated with O_2/CO_2 (19:1) in a non-recirculating system. Perfusate was pumped into the liver through the portal vein and was collected via a cannula inserted into the inferior vena cava. Effluent perfusate was channelled past a Teflon-shielded Clark-type platinum O_2 electrode and through a sampling well before being discarded. Rates of O_2 uptake by the whole organ were calculated from the difference between influent and effluent O_2 concentration, liver wet weight and perfusate flow rate.

Sodium lactate in perfusate (pH 7.4) was infused into the liver at a final concentration of 2 mM. Samples of effluent perfusate were collected and analysed for glucose, lactate and ketone bodies enzymically (Bergmeyer, 1974). Rates of metabolite production were calculated from influent minus effluent perfusate metabolite concentrations, the flow rate and the liver wet weight.

Measurement of rates of O_2 uptake in periportal and pericentral regions of the liver lobule

A miniature O_2 electrode was constructed as described by Matsumura & Thurman (1983) from 50 μm-thick platinum wire pulled under heat in a glass capillary. The electrode was coated with an acrylic ester polymer to form an O_2-permeable membrane and was used with standard polarization circuitry with an Ag/AgCl reference electrode. The electrode was used to measure O_2 partial pressure in various regions of the liver lobule by placing it on periportal (light spots) or pericentral (dark areas) regions of the liver lobule on the liver surface (Lemasters *et al.*, 1986). O_2 uptake by hepatocytes under the electrode was measured by stopping influent and effluent perfusate flow simultaneously (stopped-flow O_2-uptake technique). O_2 uptake was determined from the rate of decrease in O_2 concentration per unit time measured by the electrode (dO_2/dt) and the fluid content

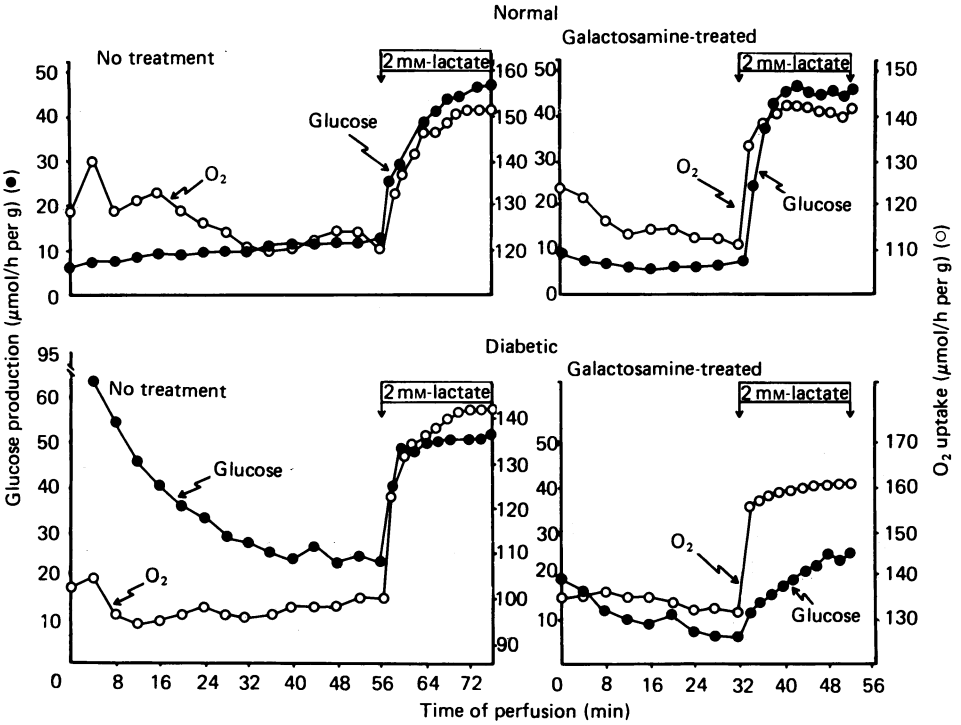

Fig. 1. Rates of glucose production and O_2 uptake by perfused livers from normal and alloxan-diabetic rats

All rats received phenobarbital in drinking water (1 g/l) from 1 to 4 weeks before injection with alloxan (120 mg/kg). Rats were treated 3 days later with galactosamine (1.5 g/kg) 3 h before liver perfusion, to inhibit glycogen synthesis (right panels), or with saline vehicle (left panels). Infusion of lactate is indicated by horizontal bars and arrows. Results of typical experiments are shown.

per g of liver, which was 0.87 ml/g under stopped-flow conditions in livers from both normal and diabetic rats (Table 1).

RESULTS

Effect of lactate on rates of glucose production and O_2 uptake by perfused livers from normal and diabetic rats

Fig. 1 shows rates of glucose production and O_2 uptake in perfused livers from normal (upper panels) and diabetic (lower panels) rats which had been pretreated with galactosamine (right panels) or vehicle (left panels). In the absence of galactosamine pretreatment, basal rates of O_2 uptake were similar in livers from control and diabetic rats. However, glucose output was very high (> 60 μmol/h per g) initially in livers from diabetic rats and declined steadily, before reaching a steady rate of around 25 μmol/h per g after 40 min of perfusion. This elevated rate of glucose output is presumably due to the high rate of glycogenolysis present in livers from alloxan-diabetic rats (Exton *et al.*, 1972). In contrast, glucose output in livers from normal rats was less than 10 μmol/h per g and was constant for up to 60 min of perfusion. When lactate was infused, both glucose production and oxygen uptake increased rapidly in livers from untreated and diabetic rats. When glucose synthesis from lactate was inhibited with 3-mercaptopicolinic acid (250 μM), an inhibitor of phosphoenolpyruvate carboxykinase (DiTullio *et al.*, 1974), O_2 uptake in all groups studied was negligible.

Galactosamine is a potent inhibitor of glycogen synthesis, which causes glycogen concentrations to decline precipitously (Decker & Keppler, 1972). After galactosamine pretreatment for 3 h, basal rates of glucose output in livers from both normal and diabetic rats were around 10 μmol/h per g (Fig. 1, right panels). Thus galactosamine treatment prevented glucose production from glycogen, but had no effect on basal rates of O_2 uptake under these conditions. Therefore all subsequent experiments in this study were performed with galactosamine-treated rats.

Glucose synthesis and O_2 uptake were measured in livers from normal and diabetic rats when lactate was increased in steps from 0.1 to 2.0 mM (Fig. 2). Stepwise increases in glucose production and O_2 uptake were observed in both groups, which returned to basal values when lactate infusion was terminated (Fig. 2). Similar titrations in livers from diabetic rats produced stepwise increases in glucose and O_2 uptake; however, maximal rates of glucose production were about 50% lower than in livers from normal rats.

Basal rates of glucose output by the whole organ were comparable (Fig. 2, Table 2) in livers from normal and diabetic rats in this study after treatment with galactosamine (< 10 μmol/h per g). When lactate was infused, however, rates of glucose output were significantly higher in livers from control (39 μmol/h per g) than from diabetic rats (25 μmol/h per g; Table 2). After lactate infusion was terminated, rates of glucose output returned to basal values in both groups.

When increases in glucose production and O_2 uptake were compared, excellent correlations ($r = 0.94$) were observed in both normal and diabetic livers (Fig. 3). However, the stoichiometry between glucose production

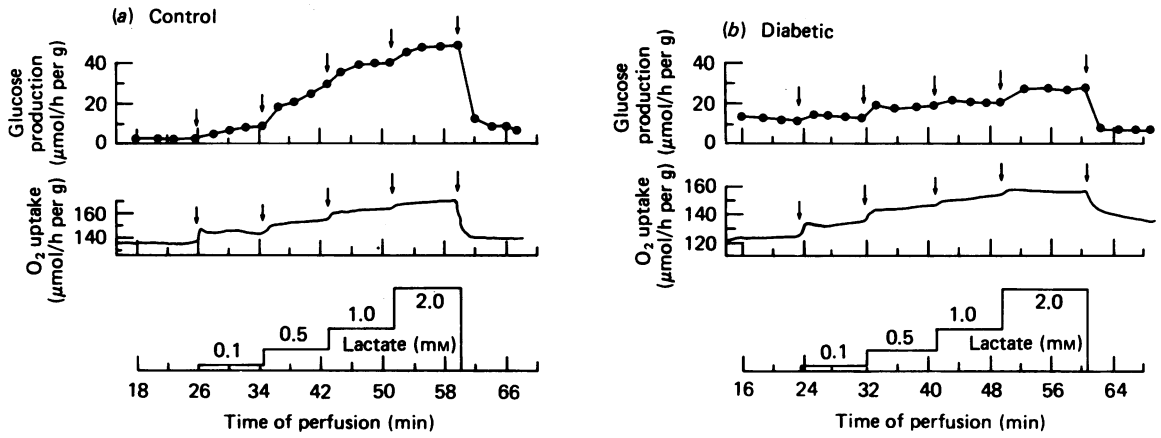

Fig. 2. Titration of gluconeogenesis and O_2 uptake with lactate in perfused livers from normal (*a*) and diabetic (*b*) rats

Phenobarbital-treated rats were starved for 24 h before experiments and were injected with galactosamine (1.5 g/kg) 3 h before perfusion. Alloxan-diabetes was induced in group *b* as described in the Materials and methods section. Livers were perfused in a non-recirculating system in the anterograde direction, and lactate was infused at the concentrations indicated in the horizontal bar (lower panel) at times denoted by arrows with a precision infusion pump. Upper panel: production of glucose. Middle panel: O_2 uptake. Rates were calculated from the influent–effluent concentration differences and the constant flow rate. Results of typical experiments are shown.

Table 2. Effect of lactate on gluconeogenesis and O_2 uptake in periportal and pericentral regions of the liver lobule in livers from normal and diabetic rats

Values are means ± S.D. for livers from six normal and five diabetic rats. Periportal and pericentral O_2 uptakes were calculated from the decrease in O_2 concentration when a miniature O_2 electrode was placed on the surface of the liver in the presence or absence of 2 mM-lactate and the flow was stopped. Whole-organ O_2 uptake was calculated from influent and effluent perfusate O_2 concentrations. Glucose output was determined enzymically in effluent perfusate samples and rates were calculated from the flow rate and liver wet wt. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for comparison with group directly above in the same column; $\dagger P < 0.01$ for comparison with treated group in normal rats.

Rats	Addition	O_2 uptake (μmol/h per g)			Glucose output (μmol/h per g)
		Periportal	Pericentral	Whole organ	
Normal	None	136±22	56±11	120±9	5.7±1.2
	Lactate	183±34**	67±7**	158±17***	38.9±6.2***
	Lactate off	131±16**	55±14*	114±10***	8.5±1.0***
Diabetic	None	162±21	73±9	133±16	6.9±3.8
	Lactate	207±23*	63±11**	157±14***	24.7±6.1***†
	Lactate off	157±35**	72±12*	122±13***	5.2±3.0***

and O_2 uptake was around 1.0 in livers from control rats, but was near 0.5 in livers from diabetic rats (Fig. 3). For an interpretation of this phenomenon, see below (the Discussion section).

Effect of lactate on O_2 uptake in periportal and pericentral regions of the liver lobule

Experiments to determine local rates of O_2 uptake were performed before, during and after infusion of lactate into perfused livers, as depicted by the hatched areas in Fig. 4. O_2 concentration was higher in periportal regions than in pericentral areas in livers from both normal and diabetic rats as a result of the natural O_2 gradient in the liver (Fig. 5). Experimental diabetes did not alter local O_2 concentrations. When perfusate flow through the liver was stopped, O_2 concentration decreased rapidly in both regions of the liver lobule; however, the rate of decrease was 2–3 times greater in periportal than in pericentral regions in livers from both groups of rats (Table 2). Subsequently, rates of O_2 uptake were calculated from the rates of change in O_2 concentration and the fluid content of the liver. Basal rates of O_2 uptake in periportal regions were not significantly different in livers from diabetic and normal rats. Basal rates of O_2 uptake were 2–3-fold higher in periportal than in pericentral regions in livers from both normal and diabetic rats, in agreement with previous studies (Matsumura *et al.*, 1984; Kinugasa & Thurman, 1986). Rates of O_2 uptake increased after lactate infusion in periportal regions in livers from both normal and diabetic rats (Table 2). In pericentral regions, basal rates of O_2 uptake were also similar in livers from normal and diabetic rats. Lactate increased the rate of O_2 uptake significantly in pericentral regions in livers from normal rats by about 10 μmol/h per g; however, it decreased O_2 uptake in livers from diabetic rats significantly by

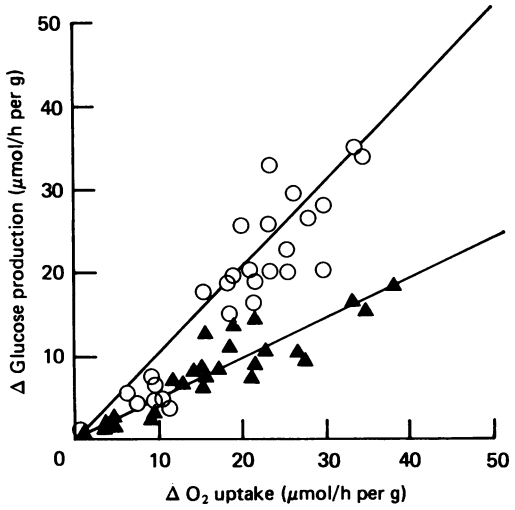

Fig. 3. Relationship between O_2 uptake and glucose production in livers from normal (○) and diabetic (▲) rats

Conditions were as in Fig. 2. The best-fit linear-regression line from data from five livers perfused at various concentrations (0.1–2.0 mM) of lactate is shown. The slope for data from normal liver was 1.03 ± 0.09, and that for data from diabetic livers was 0.48 ± 0.05 (mean ± S.E.M.): $r = 0.94$ for both.

10 μmol/h per g (Table 2). After lactate infusion was terminated, rates of O_2 uptake and glucose production returned to near-basal values in both regions of the lobule in livers from both normal and diabetic rats (Table 2).

Lactate uptake and ketone-body (β-hydroxybutyrate and acetoacetate) production were monitored also in livers from normal and diabetic rats. Lactate uptake increased as [lactate] was increased in steps in these experiments (Table 3). Maximal rates of lactate uptake were about 2-fold higher after lactate infusion (2 mM) in livers from normal than from diabetic rats. Under these conditions, rates of ketone-body production were similar in livers from both groups studied (Table 3).

DISCUSSION

Glucose synthesis in livers from galactosamine-treated rats

The lactate-induced increase in O_2 uptake observed in periportal plus pericentral regions of the normal galactosamine-treated liver can account for the glucose produced by the whole organ. In livers where endogenous long-chain fatty acids are a primary fuel, 5.4 mol of ATP is produced per mol of O_2 consumed, instead of 6 if all substrates were linked to NADH ($5.4/6.0 = 0.9$; Thurman & Scholz, 1977). Therefore, the theoretical ratio of 0.9 mol of glucose produced by gluconeogenesis per mol of O_2 consumed, and the fact that about 50% of the liver lobule is portal and about 50% is central (Lemasters *et al.*, 1986), can be used to compare local rates of glucose synthesis with measurements of glucose production by the whole organ. For example, rates of glucose synthesis by periportal regions of 47 μmol/h per g, plus 11 μmol/h per g by pericentral areas, is nearly equivalent to whole-organ rates of 33 μmol/h per g ($47/2 + 11/2 = 29$ μmol/h per g). The extra O_2 consumed

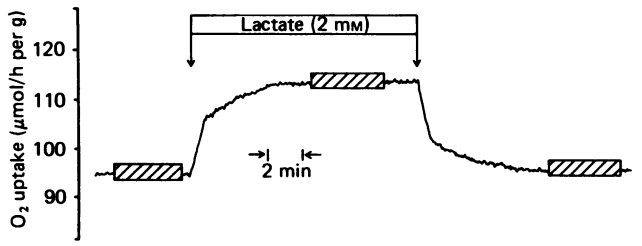

Fig. 4. Effect of lactate on the rate of O_2 uptake in perfused rat liver

O_2 partial pressure was measured with a Clark-type O_2 electrode placed in the effluent perfusate line. Livers were pre-perfused for 20 min, and stopped-flow experiments were performed before, during and after lactate infusion (hatched bars). Results of a typical experiment are shown.

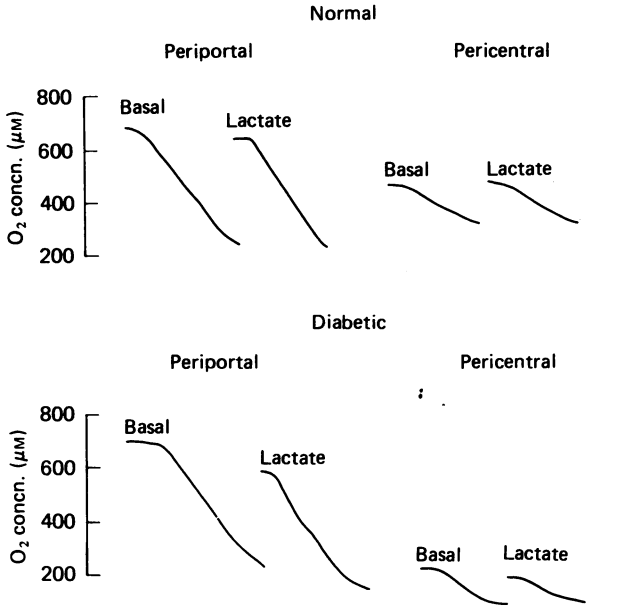

Fig. 5. Effect of stopped flow on O_2 concentration in periportal and pericentral regions of livers from normal and diabetic rats

Periportal and pericentral regions were identified on the surface of the perfused liver by their light and dark pigmentation respectively. Miniature O_2 electrodes were placed on dark or light areas on the liver surface to monitor changes in O_2 concentration after stopped flow (2–3 s) in the presence and absence of lactate (2 mM), by using the experimental protocol detailed in Fig. 4. Local rates of O_2 uptake were estimated from the tangent of the maximal decrease in O_2 concentration per unit time and the fluid volume (Table 1), as described elsewhere (Matsumura & Thurman, 1983).

owing to lactate infusion in the diabetic liver was also not far from predicted values.

In livers from galactosamine-treated control rats, the increase in O_2 uptake owing to addition of lactate occurs predominantly in periportal regions of the liver lobule (Table 2), confirming previous work with untreated rats from this laboratory (Matsumura *et al.*, 1984). A small increase in O_2 uptake also occurred in pericentral regions in livers from normal rats, consistent with previous work

Table 3. Ketone-body production and lactate uptake in livers from normal and diabetic rats

Values are means ± S.D. from four rats from the control group and six diabetic rats: $*P < 0.05$. Conditions are the same as in Fig. 2. β-Hydroxybutyrate and acetoacetate (ketone bodies) were determined enzymically from effluent perfusate samples and flow rate. Lactate uptake was calculated from the influent and effluent perfusate lactate concentrations, the flow rate and the liver wet weight.

[Lactate] (mM)	Production of β-hydroxybutyrate + acetoacetate (μmol/h per g)		Lactate uptake (μmol/h per g)	
	Normal	Diabetic	Normal	Diabetic
0	16±6	12±5		
0.1	16±6	13±6	14±3	9±7
0.5	19±6	17±4	43±13	29±20
1.0	24±6	21±3	77±38	46±18
2.0	35±4	26±3*	85±28	37±36*

(Table 2). Thus galactosamine does not appear to alter intercellular compartmentation of glucose synthesis in livers from normal rats. In livers from diabetic rats, lactate addition increased O_2 uptake to the same extent as in livers from normal rats, but only in periportal regions. Since no extra O_2 for glucose synthesis was observed in pericentral areas, it is concluded that glucose is synthesized from lactate exclusively in periportal regions of the liver lobule in diabetes. In pericentral regions, marked differences were observed between livers from normal and diabetic rats. O_2 uptake increased in livers from normal rats, but decreased in livers from diabetic rats when lactate was infused (Table 2). How can these differences be explained?

Glucose synthesis, as measured with miniature O_2 electrodes, was identical in periportal regions in livers from normal and diabetic rats (Table 2); however, lactate uptake and glucose output were markedly lower in diabetes (Tables 2 and 3). Since O_2 uptake in pericentral regions declines in livers from diabetic rats, it is concluded that some of the glucose produced in periportal regions is metabolized in pericentral regions to lactate. Metabolism of glucose to lactate would produce 2 mol of ATP per mol of glucose metabolized to lactate, thereby decreasing the O_2 requirements in pericentral regions and leading to the observed decrease in O_2 uptake in the diabetic liver (Table 2). The extra lactate produced from metabolism of glucose most likely accounts for the apparent lower rates of lactate uptake observed in the diabetic liver (Table 3).

Indeed, Katz & McGarry (1984) hypothesized that blood glucose is taken up by hepatocytes in pericentral regions of the liver and metabolized via glycolysis to lactate, which then circulates via the blood back to periportal regions where it can be converted via gluconeogenesis into glucose and ultimately into glycogen. In support of this idea, Matsumura & Thurman (1984) showed that changes in O_2 partial pressure correlated with the kinetics of glucose production after infusion of lactate, and concluded that glycolysis occurred predominantly in pericentral regions of the liver lobule. If the 'glucose paradox' hypothesis is correct, part of the glucose produced in periportal regions would not appear in the effluent perfusate in our experiments, since it was metabolized in pericentral regions. Indeed, output of glucose was about 50% less in livers from diabetic than normal rats (Table 2). Therefore, it is concluded that glucose produced in periportal areas is converted into lactate in livers from diabetic rats, data consistent with the glucose paradox hypothesis.

Potential intercellular futile cycling of carbohydrate metabolites in diabetes

The data from this study, supporting the hypothesis that glucose is synthesized from lactate in periportal regions, yet newly formed glucose is converted into lactate in pericentral areas in diabetes, is convincing. Why has this intercellular metabolism been overlooked in previous studies? The answer may be in the choice of experimental models. In previous work with isolated hepatocytes, glucose output was greater in cells from diabetic rats than in those from control rats (Wagle & Ingebretson, 1975; Lardy *et al.*, 1965). Hepatocytes are mixtures of periportal and pericentral cells. Because of the mixing of cell types from various regions, specialized functions which occur in one region of the liver lobule may have been overlooked in previous work. More recent work has demonstrated higher rates of glucose synthesis in hepatocytes isolated from periportal regions than from pericentral regions (Quistorff *et al.*, 1985); however, studies with cells isolated from specific regions of the liver lobule from diabetic rats have not yet been performed. Insight into this problem comes from the observation that specific pathways of carbohydrate metabolism follow the natural O_2 gradient in the liver. Gluconeogenesis predominates in periportal regions during perfusions in the anterograde direction, yet shifts rapidly to pericentral areas when the direction of flow (i.e. the area receiving high O_2 partial pressure) is changed (Matsumura *et al.*, 1984). Similarly, glycolysis predominates in pericentral regions of the liver lobule when flow is in the normal anterograde direction, yet predominates in periportal regions when the O_2 gradient is reversed (Matsumura & Thurman, 1983). Thus the functions of glucose synthesis or breakdown appear to be defined in some unknown manner by processes which correlate with the O_2 gradient across the liver lobule. Since hepatocytes are incubated at similar O_2 partial pressure, all cells may act similarly and intercellular metabolism may not be observed.

The concept of intercellular futile cycling was presented by Katz & McGarry (1984) as the 'glucose paradox'. Evidence for this interesting idea, however, has been scant. Why the diabetic liver carries out this energy-wasteful futile cycling of carbohydrates is unclear. It is possible that a homoeostatic mechanism dependent on glucagon or glucagon/insulin ratio has gone awry. In support of this concept, Kinugasa & Thurman (1986) observed that glucagon stimulated gluconeogenesis exclusively in periportal regions of the liver lobule in livers from normal rats. In perfusions in the retrograde direction, the effect of glucagon was shifted to pericentral regions. Thus the effect of glucagon on gluconeogenesis also followed the O_2 gradient in livers from normal rats. Gluconeogenesis may occur exclusively in periportal regions of the liver lobule in livers from diabetic rats because of the high glucagon: insulin ratio characteristic

of the diabetic state. The chronic effect of this high ratio may also explain why glucose is metabolized to lactate in pericentral regions in diabetes.

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